Zooming in on Ribosomes

Resolution Improves to 5.5 Å

The structure of the ribosome—the site for the crucial process of turning genetic code into functional proteins—is coming into focus, and the work has been advanced considerably by a team from the University of California, Santa Cruz. Working at the Macromolecular Crystallography Facility (MCF) at the ALS (Beamline 5.0.2), this team has determined the structure of the ribosome with bound messenger RNA (mRNA) and transfer RNA (tRNA) at 5.5 Å resolution. This work builds on the group's previous efforts at the MCF, in which they solved the structure to 7.8 Å. Among the new observations is structural evidence that the two main parts of the ribosome (the 30S and 50S subunits) move relative to each other during protein synthesis. The new view also offers insight into how the ribosome interacts with tRNA.

The structure was solved by using multiple-wavelength anomalous diffraction (MAD) from crystallized ribosomes of *Thermus thermophilus* bacteria. The crystals contained a synthetic mRNA analog and tRNA molecules bound to two sites (the P and E sites). The high flux from the ALS wiggler yielded diffraction data with a resolution of 5.5 Å after density modification algorithms were applied. The group additionally used ribosome complexes with and without tRNA bound to a third site (the A site) to make a Fourier difference map that showed the A-site position at 7 Å resolution.

Ribosomes consist of ribosomal RNA (rRNA) and proteins. The ribosome's ability to function is known to depend more on RNA than on protein, but until now scientists did not know why. The high-resolution structure shows the answer: the protein–protein and protein–RNA interfaces tend to occur away from functional sites, whereas the RNA–RNA interactions exist near functional centers. In addition, the interactions between the ribosome and tRNA occur mainly through contacts with rRNA.

Central to the function of the ribosome and to the revelations of this latest view of it are the intersubunit bridges. These join the two subunits, holding them together around the string of mRNA that is being decoded and the tRNA molecules whose anticodons pair with codons on the mRNA. The new crystal structure shows all the molecular components of the known contacts between the two subunits, plus two new bridges.

Previous studies have shown that the tRNAs move through the space between subunits, translocating from the A site to the P site to the E site. Now, an important structural clue to the mechanism of this motion has been glimpsed. The new structure shows that these sites are all adjacent to intersubunit bridges. Since motion occurs around these sites, and the bridges are near enough to change shape as it occurs, it is likely that this motion is coupled with movement of the subunits relative to each other. This structural information complements cryoelectron microscopy and neutron scattering studies suggesting intersubunit movement. Such studies have also made a strong case for movement of the head of the 30S subunit, relative to both the rest of that subunit and the rest of the ribosome. This is reinforced in the new structure by the finding that the four domains making up 16S rRNA are nearly structurally independent of each other (and hence can move relative to each other with little change in shape). In addition, the four domains converge near sites of functional interactions with mRNA and tRNA, suggesting that their relative movements could be closely coupled with ribosome function.

Much work remains to be done before we have a complete solution to the mystery of how the ribosome works, but this latest effort provides vital structural information against which to test models of the ribosome's machinations. ■

M.M. Yusupov (University of California, Santa Cruz; currently at CNRS, France), J.H.D. Cate (Whitehead Institute), and H.F. Noller (University of California, Santa Cruz, 831-459-2453 or -3703).

M.M. Yusupov, G.Zh. Yusupova, A. Baucom, K. Lieberman, T.N. Earnest, J.H.D. Cate, and H. F. Noller, "Crystal Structure of the Ribosome at 5.5 Å Resolution," *Science* **292**, 883 (2001).

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ZOOMING IN ON RIBOSOMES

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- **Crystal structure of *Thermus thermophilus* bacterial ribosome obtained at MCF by using multiple-wavelength anomalous diffraction (MAD)**
- **Resolution of 5.5 Å for complete 70S ribosome with bound mRNA and tRNA**
- **RNA is centrally located, near active sites; proteins are near periphery**
- **Intersubunit bridges are near tRNA binding sites**
  - *Structural evidence that subunits move relative to each other during translation*
- **Provides key structural data against which models of ribosome function can be tested**

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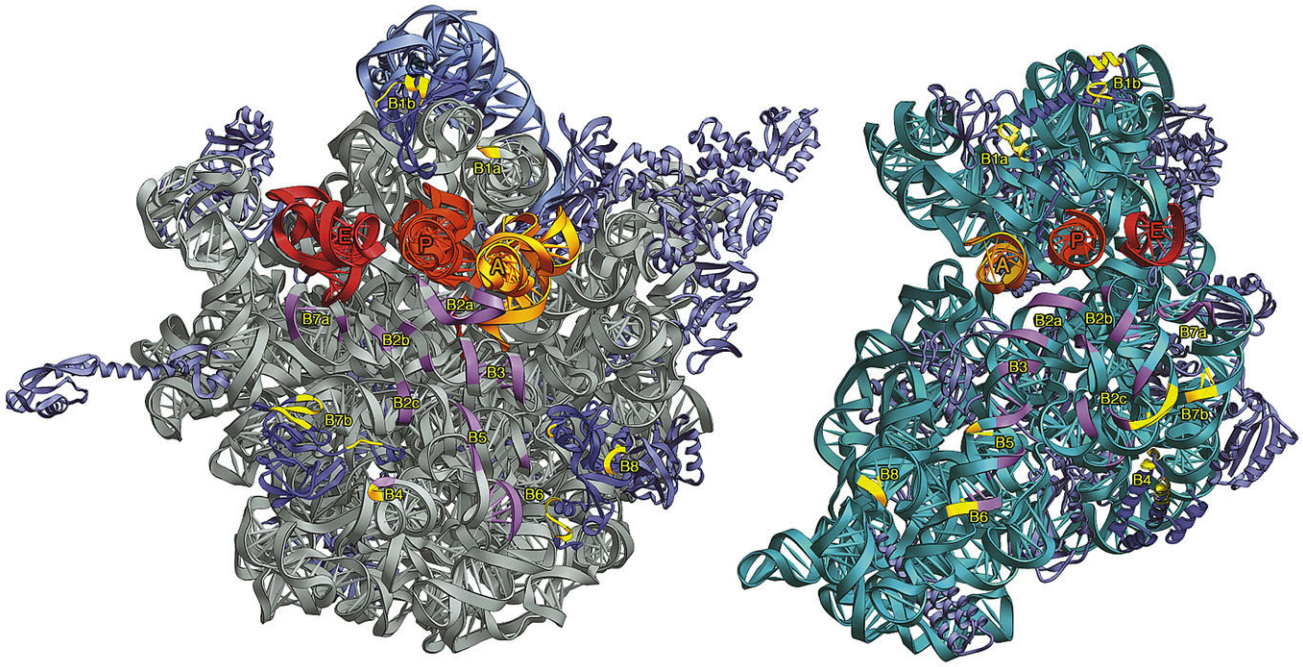

Interfaces of the 50S (left) and 30S (right) subunits of the ribosome with intersubunit bridges numbered. Magenta*, RNA–RNA contacts;* yellow*, protein–protein and protein–RNA contacts;* A, P*, and* E *mark tRNAs at left and tRNA anticodon stem loops at right.*

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